



-PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

CAHOON ET AL.

CASE NO: BB1295 US CNT

SERIAL NO: 10/690,994

GROUP ART UNIT: 1638

FILED: OCTOBER 21, 2003

EXAMINER: L. ZHENG

FOR: PLANT DIACYLGLYCEROL
ACYLTRANSFERASES

DECLARATION UNDER 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Keith R. Roesler, declare that:

I am a citizen of the United States and reside at 3928 Patricia Drive,
Urbandale, Iowa, 50322.

I am an employee of Pioneer Hi-Bred International, Inc., ("Pioneer") a
subsidiary of E.I. du Pont de Nemours and Company ("DuPont").

I received a Ph.D. in Agronomy from the University of Illinois at Urbana-
Champaign. I have worked for Pioneer from July 1, 1995, to the present in the
fields of biochemistry and molecular biology.

I am familiar with the subject matter of the above-identified application and
supervised the performance of the experiments explained below.

The following are my remarks:

1. In the December 18, 2006, Non-Final Office Action related to the above-identified application, the Examiner *inter alia* rejected claims 26-40 under 35 U.S.C. § 101 because the claimed invention is allegedly not supported by either a specific and/or substantial asserted utility or a well-established utility.
2. Described herein are a series of experiments that establish a specific and substantial asserted utility for the claimed inventions.

3. The basic experimental procedure is as follows. A *Saccharomyces cerevisiae* strain was created with two genes deleted: the *DGA1* gene, encoding acyl-CoA:diacylglycerol acyltransferase (DGAT), and the *LRO1* gene, encoding phospholipid:diacylglycerol acyltransferase (PDAT). In this yeast strain, DGAT genes were overexpressed using a strong constitutive promoter from the yeast phosphoglycerate kinase gene, and using uracil selection. DGAT assays were done using microsomal membrane preps.
4. The method of Milcamps *et al.*, 2005, "Isolation of a gene encoding a 1,2-diacylglycerol-*sn*-acetyl-CoA acetyltransferase from developing seeds of *Euonymus alatus*", J. Biol. Chem. 280:5370-5377 (attached herewith), was followed, with minor changes. *Saccharomyces cerevisiae* cultures were grown to early stationary phase in 100 ml of SC media minus uracil. Following harvest, the yeast pellets were resuspended in 4 ml of 20 mM Tris-HCl, pH 8, 10 mM MgCl₂, 1 mM EDTA, 5% glycerol, 1 mM DTT, and 0.3 M (NH₄)₂SO₄. Two ml of glass beads were added, and cells were lysed by vortexing for 5 min. The lysate was centrifuged for 15 min at 1500 g at 6 °C. The supernatant was then centrifuged at 100,000 g for 1.5 hr at 6 °C. The microsomal pellet was resuspended in 500 µl of 100 mM potassium phosphate, pH 7.2, containing 10% glycerol and frozen in liquid nitrogen prior to storage at -80 °C. Protein concentrations were determined by the method of Bradford, using the Coomassie Plus reagent (Pierce), with bovine serum albumin as standard.
5. DGAT assays were done for 1 min at 25 °C with 50 mM potassium phosphate pH 7.2, 10 µM 1-¹⁴C-labeled oleoyl-coenzyme A (50 mCi/mmol, Perkin Elmer), and 20 µg of microsomal protein, using endogenous diacylglycerol, in a total reaction volume of 100 µl. The reaction was started by addition of the microsomal membranes to the remainder of the reaction components. The assay was stopped and lipids were extracted with 2 ml of hexane:isopropanol (3:2) (Hara and Radin, 1978, "Lipid extraction of tissues with a low-toxicity solvent", Anal. Biochem. 90:420-426) containing 4 µl of unlabeled triacylglycerol (triolein, Sigma). Following vortexing for 10 sec, the phases were separated with 1 ml of 500 mM sodium sulfate and vortexing was again done for 10 sec. After 10 min, the upper phase was transferred to another tube and dried with nitrogen gas. The lipid was resolubilized in a small volume of hexane (approximately 100 to

150 µl) and applied to K6 silica TLC plates, which were developed in 80:20:1 (v/v/v) hexane:diethylether:acetic acid. Triacylglycerol was visualized and marked by staining in iodine vapor. After the stain faded, the triacylglycerol was scraped, and radioactivity was determined by liquid scintillation counting. The following results were obtained.

Construct	DGAT Activity (pmol ¹⁴ C-labeled TAG produced/min/mg microsomal protein)			
	Rep1	Rep2	Rep3	Mean ± SD
Soybean DGAT	840	887	876	868 ± 25
Wheat DGAT	518	529	515	521 ± 7
Vector control	33	14	17	21 ± 10

6. The soybean DGAT protein-coding region was obtained by PCR using clone sr1.pk0098.a8 as a template. This sequence was used to create the yeast expression vector, PHP32069 (Appendix A). The sequence of the soybean DGAT-coding region, "PHP32069 Soy DGAT1 CDS", was found to have a one nucleotide difference with the corresponding DGAT-coding region of SEQ ID NO:15, "BB1295 SEQ-15 CDS". An alignment of the two DGAT-coding sequences is presented in Appendices B1-B4. The soybean DGAT sequence in PHP32069 has an adenine (A) at position 939 of the DGAT-coding region, while the corresponding nucleotide in SEQ ID NO:15 is cytosine (C). The cDNA insert in clone sr1.pk0098.a8 was re-sequenced, and the sequence of the DGAT-coding region in sr1.pk0098.a8 was identical to the sequence of the PCR-derived DGAT-coding region in PHP32069. The single nucleotide difference in SEQ ID NO:15, also obtained by sequencing clone sr1.pk0098.a8, is presumably due to a sequencing error. This single nucleotide difference results in a one amino acid difference between the two corresponding amino acid sequences (Appendix C). The DGAT protein encoded by PHP32069 has a glutamic acid residue (E) at position 313, while the corresponding residue in SEQ ID NO:16 is aspartic acid (D).

7. The wheat DGAT DNA was obtained by a combination of PCR using clone wr1.pk0119.b6: fis as a template and a synthetic gene fragment to complete the coding region. This DGAT-coding sequence was used to create the yeast expression vector, PHP32068 (Appendix D). The sequence of this wheat DGAT-

coding region, "PHP32068 Wheat DGAT1-2 CDS", was found to have two single nucleotide differences with the corresponding DGAT-coding region of SEQ ID NO:21, "BB1295 SEQ-21 CDS". An alignment of the two DGAT-coding sequences is presented in Appendices E1-E4. The wheat DGAT nucleotide sequence of PHP32068 has a guanosine (G) at position 303 and a thymidine (T) at position 393; the sequence of SEQ ID NO:21 has a thymidine (T) and cytosine (C) at these two positions, respectively. These two nucleotide differences are "silent", i.e., the amino acid sequence encoded by PHP32068 is identical to that of SEQ ID NO:22 (Appendix F).

8. Sequence alignments were performed using the Megalign program of the LASERGENE bioinformatics computing suite (DNASTAR Inc., Madison, WI). Multiple alignment of the sequences was performed using the Clustal V method of alignment with the default parameters

9. The vector control contained no DGAT gene.

10. As shown in the table above, both the soybean DGAT protein encoded by PHP32069 and the wheat DGAT protein encoded by PHP32068 have significant DGAT activity.

11. I believe that the experiments conducted thus establish a specific and substantial utility for the claimed inventions.

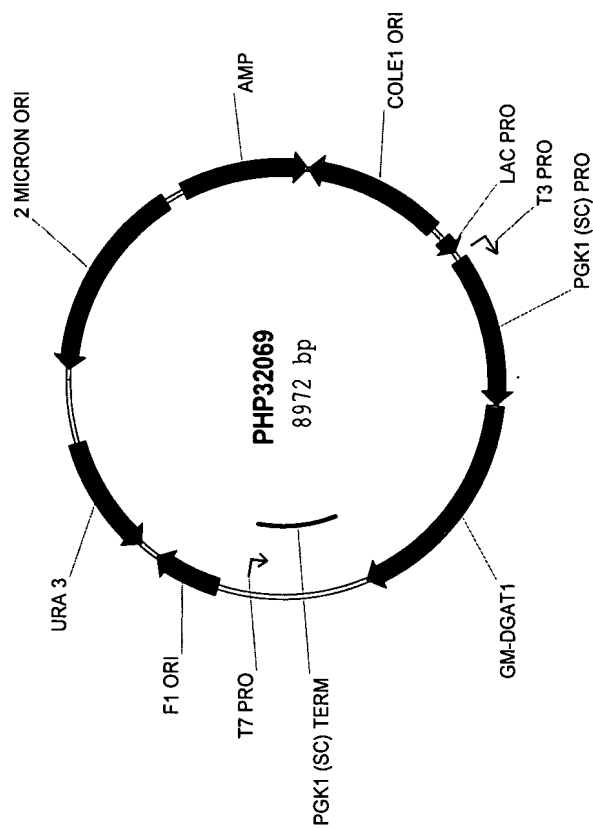
12. I declare that all statements made herein are either based on my own knowledge and are true, or if based on information and belief are believed to be true. I also declare that all statements were made with knowledge that willful false statements, and the like, are punishable by either fine, or imprisonment, or both under Section 1001 of Title 18 of the United States Code, and any such willful false statements may jeopardize the validity of either the patent application, or any patent issuing thereon.

By: Keith R. Roesler
Keith R. Roesler

Dated: 5-14-07



APPENDIX A YEAST EXPRESSION VECTOR FOR SOYBEAN DGAT



APPENDIX B1

SOYBEAN DGAT NUCLEOTIDE SEQUENCE ALIGNMENT

	A	T	G	G	C	G	A	T	T	T	C	C	G	A	T	G	A	A	A	G	T	G	T	A	G	C	C	A	C	T	G	C	T	C	T	C	A	A	C	A	C	A	C	T	C		Consensus #1			
	10																																														50			
1	A	T	G	G	C	G	A	T	T	T	C	C	G	A	T	G	A	A	A	G	T	G	T	A	G	C	C	A	C	T	G	C	T	C	T	C	A	A	C	A	C	A	C	T	C		BB1295 SEQ-15 CDS.seq			
1	A	T	G	G	C	G	A	T	T	T	C	C	G	A	T	G	A	A	A	G	T	G	T	A	G	C	C	A	C	T	G	C	T	C	T	C	A	A	C	A	C	A	C	T	C		PHP32069 Soy DGAT1 CDS.seq			
	T	T	C	C	T	G	C	G	C	C	G	C	C	T	C	C	C	A	C	C	T	C	C	A	C	C	C	C	C	C	T	C	T	C	T	C	T	C	T	C	A	A	T	T		Consensus #1				
	60																																														100			
51	T	T	C	C	T	G	C	G	C	C	C	C	G	C	C	C	T	C	C	C	C	C	T	C	A	C	C	C	C	C	C	T	C	T	C	T	C	T	C	A	A	T	T		BB1295 SEQ-15 CDS.seq					
51	T	T	C	C	T	G	C	G	C	C	C	C	C	C	C	C	T	C	C	C	C	C	T	C	A	C	C	C	C	C	C	T	C	T	C	T	C	A	A	T	T		PHP32069 Soy DGAT1 CDS.seq							
	C	G	C	C	T	G	A	G	A	C	A	C	C	A	C	G	A	C	A	G	T	T	C	C	G	G	T	G	A	T	G	A	C	T	T	G	C	C	A	A	G	A	T	T		Consensus #1				
	110																																														150			
101	C	G	C	C	T	G	A	G	A	C	A	C	C	A	C	C	A	C	A	G	T	T	C	C	G	G	T	G	A	T	G	A	C	T	T	G	C	C	A	A	G	A	T	T		BB1295 SEQ-15 CDS.seq				
101	C	G	C	C	T	G	A	G	A	C	A	C	C	A	C	C	A	C	A	G	T	T	C	C	G	G	T	G	A	T	G	A	C	T	T	G	C	C	A	A	G	A	T	T		PHP32069 Soy DGAT1 CDS.seq				
	G	G	T	T	C	C	G	A	C	T	C	C	A	T	C	A	A	C	A	G	C	G	A	C	G	C	C	G	T	C	A	A	T	T	C	C	C	A	A	T	T	C	C	A	A	C	A		Consensus #1	
	160																																														200			
151	G	G	T	T	C	C	G	A	C	T	C	C	A	T	C	A	A	C	A	G	C	A	C	G	C	G	C	G	T	C	A	A	T	T	C	C	C	A	A	T	T	C	C	A	A	C	A		BB1295 SEQ-15 CDS.seq	
151	G	G	T	T	C	C	G	A	C	T	C	C	A	T	C	A	A	C	A	G	C	A	C	G	C	G	C	G	T	C	A	A	T	T	C	C	C	A	A	T	T	C	C	A	A	C	A		PHP32069 Soy DGAT1 CDS.seq	
	G	C	A	A	A	C	G	A	C	A	A	C	A	A	C	A	C	A	C	A	G	T	T	T	C	T	C	C	G	T	C	C	A	A	T	T	C	G	C	T	C	A	A	T	T	C	C	A		Consensus #1
	210																																														250			
201	G	C	A	A	A	C	G	A	C	A	A	C	A	A	C	A	C	A	C	A	G	T	T	T	C	T	C	C	G	T	C	C	T	C	A	A	T	T	C	G	C	C	T	A	C		BB1295 SEQ-15 CDS.seq			
201	G	C	A	A	A	C	G	A	C	A	A	C	A	A	C	A	C	A	C	A	G	T	T	T	C	T	C	C	G	T	C	C	T	C	A	A	T	T	C	G	C	C	T	A	C		PHP32069 Soy DGAT1 CDS.seq			
	G	T	C	C	T	C	C	G	T	C	A	C	C	G	C	A	A	G	T	G	A	A	G	T	G	A	A	G	T	C	C	G	C	T	C	A	A	T	T	C	A	G	C	T	C		Consensus #1			
	260																																														300			
251	G	T	C	C	T	C	C	G	T	C	A	C	C	G	C	T	C	A	C	C	G	C	A	A	G	T	G	A	A	G	T	C	C	G	C	T	C	A	G	C	T	C		BB1295 SEQ-15 CDS.seq						
251	G	T	C	C	T	C	C	G	T	C	A	C	C	G	C	T	C	A	C	C	G	C	A	A	G	T	G	A	A	G	T	C	C	G	C	T	C	A	G	C	T	C		PHP32069 Soy DGAT1 CDS.seq						
	G	A	C	A	C	T	A	T	T	T	C	C	G	T	C	A	G	A	G	T	C	A	C	C	G	G	C	C	T	T	C	A	C	C	T	T	C	A	C	T	T	G	T	A	T		Consensus #1			
	310																																														350			
301	G	A	C	A	C	T	A	T	T	T	C	C	G	T	C	A	G	A	G	T	C	A	C	C	G	G	C	C	T	T	C	A	A	C	C	T	T	G	T	A	T	A	T		BB1295 SEQ-15 CDS.seq					
301	G	A	C	A	C	T	A	T	T	T	C	C	G	T	C	A	G	A	G	T	C	A	C	C	G	G	C	C	T	T	C	A	A	C	C	T	T	G	T	A	T	A	T		PHP32069 Soy DGAT1 CDS.seq					
	A	G	T	C	C	T	T	G	C	T	G	T	G	A	T	A	G	C	C	G	A	C	T	C	A	T	T	G	A	G	A	T	T	A	T	G	A	T	A	T	G	A	T		Consensus #1					
	360																																														400			
351	A	G	T	C	C	T	T	G	C	T	T	G	T	G	A	T	A	G	C	C	A	C	T	C	A	T	T	T	G	A	G	A	T	T	A	T	G	A	T	A	T	G	A	T		BB1295 SEQ-15 CDS.seq				
351	A	G	T	C	C	T	T	G	C	T	T	G	T	G	A	T	A	G	C	C	A	C	T	C	A	T	T	T	G	A	G	A	T	T	A	T	G	A	T	A	T	G	A	T		PHP32069 Soy DGAT1 CDS.seq				

APPENDIX B2

	A	T	G	G	T	G	T	G	A	T	C	T	G	G	C	T	T	T	G	G	T	T	T	A	G	T	T	C	A	A	G	T	C	A	T	T	G	A	C	A		Consensus #1					
	410																																								450						
401	A	T	G	G	T	G	G	T	G	A	T	C	A	A	T	C	T	T	G	G	C	T	T	T	A	G	T	T	C	A	A	G	T	C	A	T	T	G	A	C	A		BB1295 SQ-15 CDS.seq				
401	A	T	G	G	T	G	G	T	G	A	T	C	A	A	T	C	T	T	T	G	G	C	T	T	T	A	G	T	T	C	A	A	G	T	C	A	T	T	G	A	C	A		PHF32069 Soy DGAT1 CDS.seq			
	420																																								430						
	430																																								440						
	440																																								450						
	450																																								500						
	460																																								510						
451	G	A	C	T	G	G	C	C	C	T	T	T	C	A	T	G	T	G	T	G	T	T	C	T	C	T	C	T	T	G	T	A	T	T	C	C	T	T	C	C		BB1295 SQ-15 CDS.seq					
451	G	A	C	T	G	G	C	C	C	T	T	T	C	A	T	G	T	G	T	G	T	T	C	T	C	T	C	T	T	G	T	A	T	T	C	C	T	T	C	C		PHF32069 Soy DGAT1 CDS.seq					
	470																																								520						
	480																																								530						
	490																																								540						
	500																																								550						
501	T	G	C	C	T	T	A	T	A	G	T	G	G	A	G	A	G	T	T	G	G	C	A	C	A	C	A	A	G	T	G	T	A	T	A	C	C	C	G	A	C	C	A		Consensus #1		
	510																																								560						
501	T	G	C	C	T	T	A	T	A	G	T	G	G	A	G	A	G	T	T	G	G	C	A	C	A	C	A	A	G	T	G	T	A	T	A	C	C	C	G	A	C	C	A		BB1295 SQ-15 CDS.seq		
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	530																																								580						
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	550																																								600						
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	560																																								610						
	570																																								620						
	580																																								630						
	590																																								640						
	600																																								650						
601	G	T	T	A	G	T	T	A	C	T	C	A	G	G	T	G	T	T	T	G	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T		BB1295 SQ-15 CDS.seq		
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	610																																								660						
	620																																								670						
	630																																								680						
	640																																								690						
	650																																								700						
651	A	A	T	G	C	T	G	T	T	C	T	T	G	T	G	T	T	A	A	A	A	T	T	G	G	T	G	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T		Consensus #1			
651	A	A	T	G	C	T	G	T	T	C	T	T	G	T	G	T	T	A	A	A	A	T	T	G	G	T	G	T	T	T	T	T	T	T	T	T	T	T	T	T	T		BB1295 SQ-15 CDS.seq				
	660																																								710						
	670																																								720						
	680																																								730						
	690																																								740						
	700																																								750						
701	C	A	A	C	T	A	T	G	A	G	A	G	C	A	C	T	T	A	C	C	A	A	T	T	A	G	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T		BB1295 SQ-15 CDS.seq				
701	C	A	A	C	T	A	T	G	A	G	A	G	C	A	C	T	T	A	C	C	A	A	T	T	A	G	T	T	T	T	T	T	T	T	T	T	T	T	T	T		PHF32069 Soy DGAT1 CDS.seq					
	710																																								760						
	720																																								770						
	730																																								780						
	740																																								790						
	750																																								800						
751	C	T	G	C	T	C	G	A	T	A	C	T	C	T	G	A	C	T	T	A	C	C	T	T	A	C	A	C	T	T	T	T	T	T	T	T	T	T	T	T	T		BB1295 SQ-15 CDS.seq				
751	C	T	G	C	T	C	G	A	T	A	C	T	C	T	G	A	C	T	T	A	C	C	T	T	A	C	A	C	T	T	T	T	T	T	T	T	T	T	T	T		PHF32069 Soy DGAT1 CDS.seq					

APPENDIX B3

SOYBEAN DGAT NUCLEOTIDE SEQUENCE ALIGNMENT

[illegible]

APPENDIX B4

[illegible]

Consensus 'Consensus #1': When all match the residue of BB1295 SEQ-15 CDS.seq show the residue of BB1295 SEQ-15 CDS.seq, otherwise show '.'.

Decoration 'Decoration #1': Box residues that differ from BB1295 SEQ-15 CDS.seq.

APPENDIX C

SOYBEAN DGAT AMINO ACID SEQUENCE ALIGNMENT

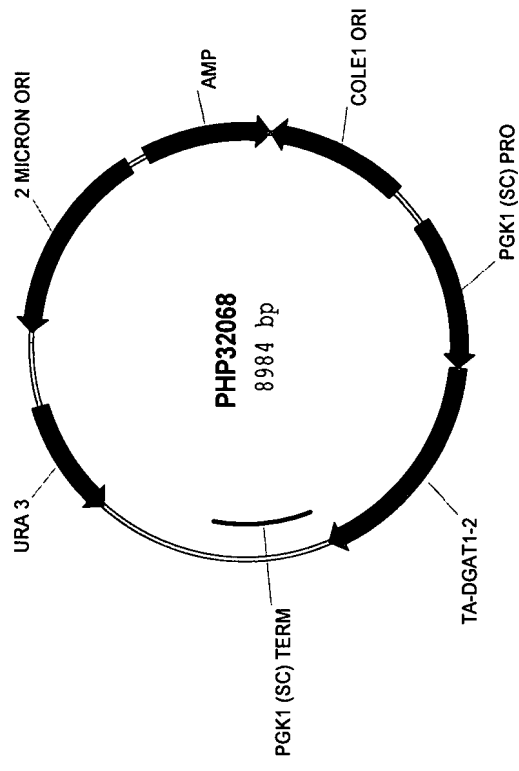
	M A I S D E P E S V A T A L N H S S L R R R P S A T S T A G L F N S P E T T T D S S C D D L A K D S G S D S I N S D D	Consensus #1
	10 20 30 40 50 60	
1	M A I S D E P E S V A T A L N H S S L R R R P S A T S T A G L F N S P E T T T D S S C D D L A K D S G S D S I N S D D	Bt1295 USCNT SEQ-16.pro
1	M A I S D E P E S V A T A L N H S S L R R R P S A T S T A G L F N S P E T T T D S S C D D L A K D S G S D S I N S D D	PHP32069 Soy DGAT1.pro
	A A V N S Q Q O N E K Q D T D F S V L K F A Y R P S V P A H R K V K E S P L S S D T I F R O S H A G L F N L C I V V L V	Consensus #1
	70 80 90 100 110 120	
61	A A V N S Q Q O N E K Q D T D F S V L K F A Y R P S V P A H R K V K E S P L S S D T I F R Q S H A G L F N L C I V V L V	Bt1295 USCNT SEQ-16.pro
61	A A V N S Q Q O N E K Q D T D F S V L K F A Y R P S V P A H R K V K E S P L S S D T I F R Q S H A G L F N L C I V V L V	PHP32069 Soy DGAT1.pro
	A V N S R L I I E N L M K Y G W L I K S G F W F S S K S L R D W P L F M C C L S L V V P F F A A F I V E K L A Q R K C I	Consensus #1
	130 140 150 160 170 180	
121	A V N S R L I I E N L M K Y G W L I K S G F W F S S K S L R D W P L F M C C L S L V V P F F A A F I V E K L A Q R K C I	Bt1295 USCNT SEQ-16.pro
121	A V N S R L I I E N L M K Y G W L I K S G F W F S S K S L R D W P L F M C C L S L V V P F F A A F I V E K L A Q R K C I	PHP32069 Soy DGAT1.pro
	P E P V V V V L H I I I T S T S L F Y P V L V I L R C D S A F V S G V T L M L F S C V V W L K L V S Y A H T N Y D M R A	Consensus #1
	190 200 210 220 230 240	
181	P E P V V V V L H I I I T S T S L F Y P V L V I L R C D S A F V S G V T L M L F S C V V W L K L V S Y A H T N Y D M R A	Bt1295 USCNT SEQ-16.pro
181	P E P V V V V L H I I I T S T S L F Y P V L V I L R C D S A F V S G V T L M L F S C V V W L K L V S Y A H T N Y D M R A	PHP32069 Soy DGAT1.pro
	L T K L V E K G E A L L D T L N M D Y P Y N V S F K S L A Y F L V A P T L C Y Q P S Y P R T P Y I I R K G W L F R Q L V K	Consensus #1
	250 260 270 280 290 300	
241	L T K L V E K G E A L L D T L N M D Y P Y N V S F K S L A Y F L V A P T L C Y Q P S Y P R T P Y I I R K G W L F R Q L V K	Bt1295 USCNT SEQ-16.pro
241	L T K L V E K G E A L L D T L N M D Y P Y N V S F K S L A Y F L V A P T L C Y Q P S Y P R T P Y I I R K G W L F R Q L V K	PHP32069 Soy DGAT1.pro
	L I I F T G V M G F I I - Q Y I N P I V Q N S Q H P L K G N L L Y A T E R V L K L S V P N L Y V W L C M F Y C F F H L W	Consensus #1
	310 320 330 340 350 360	
301	L I I F T G V M G F I I D Q Y I N P I V Q N S Q H P L K G N L L Y A T E R V L K L S V P N L Y V W L C M F Y C F F H L W	Bt1295 USCNT SEQ-16.pro
301	L I I F T G V M G F I I [E] Q Y I N P I V Q N S Q H P L K G N L L Y A T E R V L K L S V P N L Y V W L C M F Y C F F H L W	PHP32069 Soy DGAT1.pro
	L N I A E L L R F G D R E F Y K D W W N A K T V E D Y W R M W N M P V H K W M I R H L Y F P C L R H G L P K A A L L	Consensus #1
	370 380 390 400 410 420	
361	L N I A E L L R F G D R E F Y K D W W N A K T V E D Y W R M W N M P V H K W M I R H L Y F P C L R H G L P K A A L L	Bt1295 USCNT SEQ-16.pro
361	L N I A E L L R F G D R E F Y K D W W N A K T V E D Y W R M W N M P V H K W M I R H L Y F P C L R H G L P K A A L L	PHP32069 Soy DGAT1.pro
	I A F L V S A L F H E L C I A V P C H I F K L W A F G G I M F Q V P L V I T N Y L Q N K F R N S M V G N M I F W F I F	Consensus #1
	430 440 450 460 470 480	
421	I A F L V S A L F H E L C I A V P C H I F K L W A F G G I M F Q V P L V I T N Y L Q N K F R N S M V G N M I F W F I F	Bt1295 USCNT SEQ-16.pro
421	I A F L V S A L F H E L C I A V P C H I F K L W A F G G I M F Q V P L V I T N Y L Q N K F R N S M V G N M I F W F I F	PHP32069 Soy DGAT1.pro
	S I L G Q P M C V L L Y Y H D L M N R K K G K L D	Consensus #1
	490 500	
481	S I L G Q P M C V L L Y Y H D L M N R K K G K L D	Bt1295 USCNT SEQ-16.pro
481	S I L G Q P M C V L L Y Y H D L M N R K K G K L D	PHP32069 Soy DGAT1.pro

Consensus 'Consensus #1': When all match the residue of BB1295 USCNT SEQ-16.pro show the residue of BB1295 USCNT SEQ-16.pro, otherwise show '.'

Decoration 'Decoration #1': Box residues that differ from BB1295 USCNT SEQ-16.pro.

APPENDIX D

YEAST EXPRESSION VECTOR FOR WHEAT DGAT



APPENDIX E1
WHEAT DGAT NUCLEOTIDE SEQUENCE ALIGNMENT

	A	T	G	T	C	A	A	A	G	G	A	A	C	C	C	A	G	A	C	C	C	G	C	A	G	C	T	T	C	C	T	T	C		Consensus #1														
	10									20								30																	40										50				
1	A	T	G	T	C	A	A	A	G	G	A	A	C	C	C	A	G	A	C	C	C	G	G	C	A	G	C	T	T	C	C	T	T	C	C	T	T	C	C	T	T	C	C	T	T	C		BB1295 SEQ-21 CDS.seq	
1	A	T	G	T	C	A	A	A	G	G	A	A	C	C	C	A	G	A	C	C	C	G	G	C	A	G	C	T	T	C	C	T	T	C	C	T	T	C	C	T	T	C	C	T	T	C		PHP32068 Wheat DGAT1-2 CDS.seq	
	C	C	A	C	G	G	C	G	C	C	A	C	C	A	A	A	C	C	C	C	C	C	C	C	A	A	C	C	T	T	C	C	G	A	A	C	T	T	C	C	G	G	A		Consensus #1				
	60									70								80																	90										100				
51	C	C	A	C	G	G	C	G	C	C	A	C	C	A	A	A	C	C	C	C	C	C	C	C	C	C	A	A	C	C	T	T	C	C	G	A	A	C	C	T	T	C	C	G	A		BB1295 SEQ-21 CDS.seq		
51	C	C	A	C	G	G	C	G	C	C	A	C	C	A	A	A	C	C	C	C	C	C	C	C	C	C	A	A	C	C	T	T	C	C	G	A	A	C	C	T	T	C	C	G	A		PHP32068 Wheat DGAT1-2 CDS.seq		
	A	C	C	T	C	C	T	C	A	G	T	T	C	C	A	C	C	A	T	G	G	C	C	C	C	C	T	C	G	T	T	C	G	C	T	T	C	G	C	T	T	C	G	C	T		Consensus #1		
	110									120								130																	140												150		
101	A	C	C	T	C	C	T	C	A	G	T	T	C	C	A	C	C	C	C	C	C	C	C	C	C	C	T	T	C	G	C	T	T	C	G	C	C	T	T	C	G	C	T	T	C		BB1295 SEQ-21 CDS.seq		
101	A	C	C	T	C	C	T	C	A	G	T	T	C	C	A	C	C	A	T	G	G	C	C	C	C	C	C	T	T	C	G	C	T	T	C	G	C	T	T	C	G	C	T	T	C		PHP32068 Wheat DGAT1-2 CDS.seq		
	A	C	G	A	T	C	G	C	A	C	A	C	C	C	T	C	C	C	T	C	G	C	C	C	C	C	C	T	T	C	G	C	C	C	C	T	T	C	G	C	C	C	G	A		Consensus #1			
	160									170								180																	190												200		
151	A	C	G	A	T	C	G	C	A	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	T	T	C	G	C	C	C	C	C	T	T	C	G	C	C	C	G	A		BB1295 SEQ-21 CDS.seq			
151	A	C	G	A	T	C	G	C	A	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	T	T	C	G	C	C	C	C	C	T	T	C	G	C	C	C	G	A		PHP32068 Wheat DGAT1-2 CDS.seq			
	G	G	T	C	C	A	C	G	A	G	G	C	G	C	A	T	G	A	G	C	A	G	C	C	C	C	C	A	C	G	A	C	G	A	C	G	A	C	G	A	T	G		Consensus #1					
	210									220								230																	240												250		
201	G	G	T	C	C	A	C	G	A	G	G	C	G	C	C	A	T	G	A	G	C	A	G	C	C	C	C	C	C	C	C	C	C	C	C	A	C	C	A	C	G	A	C	G	A	T	G		BB1295 SEQ-21 CDS.seq
201	G	G	T	C	C	A	C	G	A	G	G	C	G	C	C	A	T	G	A	G	C	A	G	C	C	C	C	C	C	C	C	C	C	C	C	A	C	C	A	C	G	A	C	G	A	T	G		PHP32068 Wheat DGAT1-2 CDS.seq
	C	C	C	T	G	C	T	A	C	C	G	G	C	G	T	C	G	G	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	A	A	G	A	G	A	G	C	C	G		Consensus #1		
	260									270								280																	290												300		
251	C	C	C	T	G	C	T	A	C	C	G	G	C	G	T	C	G	C	G	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	A	A	G	A	G	A	G	C	C	G		BB1295 SEQ-21 CDS.seq		
251	C	C	C	T	G	C	T	A	C	C	G	G	C	G	T	C	G	C	G	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	A	A	G	A	G	A	G	C	C	G		PHP32068 Wheat DGAT1-2 CDS.seq		
	C	T	.	A	G	C	T	C	C	G	A	C	G	C	A	T	C	T	C	G	A	C	A	G	C	C	A	T	G	C	A	T	T	C	T	T	C	T	T	C	T	T	C	T	T	C		Consensus #1	
	310									320								330																	340												350		
301	C	T	.	A	G	C	T	C	C	G	A	C	G	C	A	T	C	T	C	G	A	C	A	G	C	C	A	T	G	C	A	T	T	C	T	T	C	T	T	C	T	T	C	T	T	C		BB1295 SEQ-21 CDS.seq	
301	C	T	[G]	A	G	C	T	C	C	G	A	C	G	C	A	T	C	T	C	G	A	C	A	G	C	C	A	T	G	C	A	T	T	C	T	T	C	T	T	C	T	T	C	T	T	C		PHP32068 Wheat DGAT1-2 CDS.seq	
	A	T	G	C	A	T	T	G	T	G	C	T	G	A	T	T	G	C	A	G	T	G	A	C	A	G	C	T	C	A	T	T	A	T	.	G	A	G	A	A	C	T		Consensus #1					
	360									370								380																	390												400		
351	A	T	G	C	A	T	T	G	T	G	C	T	G	A	T	T	G	C	A	G	T	G	A	C	A	G	C	T	C	A	T	T	C	T	T	C	T	T	C	T	T	C	T	T	C		BB1295 SEQ-21 CDS.seq		
351	A	T	G	C	A	T	T	G	T	G	C	T	G	A	T	T	G	C	A	G	T	G	A	C	A	G	C	T	C	A	T	T	[T]	T	T	G	A	G	A	A	C	T		PHP32068 Wheat DGAT1-2 CDS.seq					

APPENDIX E2

WHEAT DGAT NUCLEOTIDE SEQUENCE ALIGNMENT

	T	A	T	G	A	A	G	T	A	T	T	A	A	T	A	A	G	A	G	C	T	G	G	T	T	T	G	G	T	T	T	A	G	T	G	C	A	A	G	A		Consensus #1		
	410												420												430																		450	
401	T	A	T	G	A	A	G	T	A	T	T	A	A	T	A	A	G	A	G	C	T	G	G	T	T	T	G	G	T	T	T	A	G	T	G	C	A	A	G	A		BB1295 SEQ-21 CDS.seq		
401	T	A	T	G	A	A	G	T	A	T	T	A	A	T	A	A	G	A	G	C	T	G	G	T	T	T	G	G	T	T	T	A	G	T	G	C	A	A	G	A		PHP32068 Wheat DGAT1-2 CDS.seq		
																																										Consensus #1		
	460												470												480																		500	
451	T	C	G	C	T	G	G	C	C	A	T	T	C	T	G	A	T	G	T	G	T	G	C	T	C	A	C	T	T	T	A	C	C	A	T	T	T	T	T	T		BB1295 SEQ-21 CDS.seq		
451	T	C	G	C	T	G	G	C	C	A	T	T	C	T	G	A	T	G	T	G	C	T	G	C	T	C	A	C	T	T	T	A	C	C	A	T	T	T	T		PHP32068 Wheat DGAT1-2 CDS.seq			
																																										Consensus #1		
	510												520												530																		550	
501	C	C	A	C	T	G	C	T	C	T	C	A	T	G	A	C	C	G	A	G	A	G	T	G	G	C	T	C	A	A	A	A	G	C	T	C	A	T	C		BB1295 SEQ-21 CDS.seq			
501	C	C	A	C	T	G	C	T	C	T	C	A	T	G	A	C	C	G	A	G	A	G	T	G	G	C	T	C	A	A	A	A	G	C	T	C	A	T	C		PHP32068 Wheat DGAT1-2 CDS.seq			
																																										Consensus #1		
	560												570												580																		600	
551	G	T	G	A	T	C	A	T	G	T	C	T	C	C	A	T	A	T	A	T	A	T	A	T	T	A	C	A	C	C	A	C	T	G	T	C	C	T	T		BB1295 SEQ-21 CDS.seq			
551	G	T	G	A	T	C	A	T	G	T	C	T	C	C	A	T	A	T	A	T	A	T	A	T	T	A	C	A	C	C	A	C	T	G	T	C	C	T	T		PHP32068 Wheat DGAT1-2 CDS.seq			
																																										Consensus #1		
	610												620												630																		650	
601	A	T	C	T	A	T	C	C	G	T	T	G	T	G	A	T	T	C	T	A	A	G	T	G	T	G	A	T	C	A	G	C	A	G	T	A	T	A	T	C	T	G		
601	A	T	C	T	A	T	C	C	G	T	T	G	T	G	A	T	T	C	T	A	A	G	T	G	T	G	A	T	C	A	G	C	A	G	T	A	T	A	T	C	T	G		
																																										Consensus #1		
	660												670												680																		700	
651	A	T	T	G	T	G	T	T	A	A	T	G	T	T	C	A	T	T	G	C	A	A	G	C	A	T	T	G	A	G	C	T	T	G	A	A	G	C	T	T	T			
651	A	T	T	G	T	G	T	T	A	A	T	G	T	T	C	A	T	T	G	C	A	A	G	C	A	T	T	G	A	G	C	T	T	G	A	A	G	C	T	T	T			
																																										Consensus #1		
	710												720												730																		750	
701	T	T	G	C	T	C	A	T	A	C	A	A	T	T	A	T	A	A	G	G	A	T	A	T	T	T	G	T	C	C	A	A	A	G	T	A	T	T	G	A	A	A	G	
701	T	T	G	C	T	C	A	T	A	C	A	A	T	T	A	T	A	A	G	G	A	T	A	T	T	T	G	T	C	C	A	A	A	G	T	A	T	T	G	A	A	A	G	
																																										Consensus #1		
	760												770												780																		800	
751	G	G	T	G	C	T	A	C	A	C	A	T	G	G	C	A	G	T	T	C	T	A	T	C	G	A	T	G	A	G	A	A	A	A	C	A	T	A	A	G	C	C	A	C
751	G	G	T	G	C	T	A	C	A	C	A	T	G	G	C	A	G	T	T	C	T	A	T	C	G	A	T	G	A	G	A	A	A	A	C	A	T	A	A	G	C	C	A	C

APPENDIX E3

1151

APPENDIX E4

WHEAT DGAT NUCLEOTIDE SEQUENCE ALIGNMENT

	A A G T G G A T C G G T T C G A C A T A T A T A T T T T C C A T G C A T A A G G A A T G G C T T A T C	1210	1220	1230	1240	1250	Consensus #1
1201	A A G T G G A T C G G T T C G A C A T A T A T T T T C C A T G C A T A A G G A A T G G C T T A T C						BB1295 SEQ-21 CDS.seq
1201	A A G T G G A T C G G T T C G A C A T A T A T T T T C C A T G C A T A A G G A A T G G C T T A T C						PHP32068 Wheat DGAT1-2 CDS.seq
	A A A G G G T G T G C C A T T C T C A T C G C C A T T T C T G G T T C A G C T G T A T T T C A T G	1260	1270	1280	1290	1300	Consensus #1
1251	A A A G G G T G T G C C A T T C T C A T C G C C A T T T C T G G T T C A G C T G T A T T T C A T G						BB1295 SEQ-21 CDS.seq
1251	A A A G G G T G T G C C A T T C T C A T C G C C A T T T C T G G T T C A G C T G T A T T T C A T G						PHP32068 Wheat DGAT1-2 CDS.seq
	A G C T A T G T A T T G C T G T C C G T G C C A C A T T T T C A A A T T A T G G G C A T T T C T	1310	1320	1330	1340	1350	Consensus #1
1301	A G C T A T G T A T T G C T G T C C G T G C C A C A T T T T C A A A T T A T G G G C A T T T C T						BB1295 SEQ-21 CDS.seq
1301	A G C T A T G T A T T G C T G T C C G T G C C A C A T T T T C A A A T T A T G G G C A T T T C T						PHP32068 Wheat DGAT1-2 CDS.seq
	G G A A T C A T G T T T C A G A T T C C C C T G C T A T T C T T G A C G A A A T A T C T T C A A G A	1360	1370	1380	1390	1400	Consensus #1
1351	G G A A T C A T G T T T C A G A T T C C C C T G C T A T T C T T G A C G A A A T A T C T T C A A G A						BB1295 SEQ-21 CDS.seq
1351	G G A A T C A T G T T T C A G A T T C C C C T G C T A T T C T T G A C G A A A T A T C T T C A A G A						PHP32068 Wheat DGAT1-2 CDS.seq
	T A A G T T C A A G A A T A C A A T G G T G G G C A C A T G A T A T T T G G T T C T T C T C A	1410	1420	1430	1440	1450	Consensus #1
1401	T A A G T T C A A G A A T A C A A T G G T G G G C A C A T G A T A T T T G G T T C T T C T T C A						BB1295 SEQ-21 CDS.seq
1401	T A A G T T C A A G A A T A C A A T G G T G G G C A C A T G A T A T T T G G T T C T T C T T C A						PHP32068 Wheat DGAT1-2 CDS.seq
	G C A T A G T G G G C A C C A A T G T G T G T T C T C T T G T A C T A C C A T G A T G T C A T G	1460	1470	1480	1490	1500	Consensus #1
1451	G C A T A G T G G G C A C C A A T G T G T G T T C T C T T G T A C T A C C A T G A T G T C A T G						BB1295 SEQ-21 CDS.seq
1451	G C A T A G T G G G C A C C A A T G T G T G T T C T C T T G T A C T A C C A T G A T G T C A T G						PHP32068 Wheat DGAT1-2 CDS.seq
	A C A G A C A G G C T C A G A C A A T G G C	1510	1520				Consensus #1
1501	A C A G A C A G G C T C A G A C A A T G G C						BB1295 SEQ-21 CDS.seq
1501	A C A G A C A G G C T C A G A C A A T G G C						PHP32068 Wheat DGAT1-2 CDS.seq

Consensus 'Consensus #1': When all match the residue of BB1295 SEQ-21 CDS.seq show the residue of BB1295 SEQ-21 CDS.seq, otherwise show '.,'.D

Decoration 'Decoration #1': Box residues that differ from BB1295 SEQ-21 CDS.seq.

